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HLA Class II Alleles in Ainu Living in Hidaka District, Hokkaido, Northern Japan

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ABSTRACT The Ainu people are considered to be the descendants of preagricultural native populations of northern Japan, while the majority of the population of contemporary Japan (Wajin) is descended mainly from postneolithic migrants. Polymorphisms of the HLA-DRB1, DRB3, and DQB1 alleles were investigated in DNA samples of 50 Ainu living in Hidaka district, Hokkaido. Unique features of the Ainu in this study were high incidences of DRB1*1401, DRB1*1406, and a newly described allele, DRB1*1106 (20%, 17%, and 5%, respectively). On the other hand, several common alleles in Wajin (DRB1*1502, 1302, 0803, and 1501) were found at relatively low frequencies (1–2%) in Ainu. Previously DRB1*1406 was described as a characteristic allele of some Native American or northeast Asian ethnic groups, and DRB1*1106 had been found in only two Singapore Chinese and one Korean. Principal component analysis of various populations based on HLA class II allele frequencies places the Ainu population midway between other east Asian populations, including Wajin, and Native Americans. These observations may support the hypothesis that the Ainu people are the descendants of some Upper Paleolithic populations of northeast Asia from which Native Americans are also descended.

The Ainu people of Hokkaido, the northernmost island of Japan, are considered to have descended from the original population of northern Japan. Although earlier anthropological observations based on morphological features had claimed that the Ainu are so-called Caucasoids, recent genetic and morphological investigations have indicated their close relationship to so-called Mongoloid populations (Omoto, 1972; Misawa et al., 1975; Harihara et al., 1986).

More than 20 years ago, Mittal et al. (1973) reported observing HLA (human leukocyte antigens) class I (HLA-A and B) polymorphism in the Ainu based on serological typing. However, in that study neither serological split antigens nor HLA class II antigens were analyzed due to methodological

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limitations. In the present study, we investigated the polymorphisms of the HLA class II genes and haplotypes in the Ainu by means of DNA-based high-resolution typing.

Although HLA antigens play a key role in the recognition of foreign antigens, there is evidence that each haplotype has been well conserved during human evolution (Tokunaga et al., 1988). Because of these features, HLA alleles and haplotypes serve as powerful markers in the field of human population genetics (Tokunaga et al., 1996). Accordingly, we also compare the similarity of Ainu to a number of Asian and American populations.

MATERIALS AND METHODS

Populations

There are approximately 20,000 Ainu in Japan. Most of them are more or less mixed even in their homeland, Hokkaido. Groups exhibiting morphological as well as cultural features ascribed exclusively to Ainu exist only in some areas, including the Hidaka district of Hokkaido. The Ainu DNA samples analyzed in this study are the same as those analyzed in the mitochondrial (mt) DNA study by Harihara et al. (1986). Genomic DNAs were extracted from the blood samples obtained from 50 Ainu adults living in the Hidaka district, Hokkaido. No pedigree data were available, and the admixture rates were unknown.

HLA class II antigens

The HLAs of class II (DR, DQ, and DP) are heterodimers (α and β chains) of highly polymorphic glycoproteins expressed on the surface of antigen-presenting cells of the immune system. The α and β chains of class II antigens are encoded separately by genes situated close together in the MHC (major histocompatibility complex) region on chromosome 6. DRα, DRβ, DQα, and DQβ molecules are encoded by the DRA, DRB, DQA1, and DQB1 genes, respectively. The β chain of HLA-DR antigens shows a remarkably high degree of polymorphism in the first domain, which is encoded by the second exon of HLA-DRB genes. Several functional HLA-DRB genes, DRB1, B3, B4, and B5, have been described and thus far reported to possess 124, four, five, and five alleles, respectively (Bodmer et al., 1995). All HLA-DR types have the DRB1 gene, but possession of the DRB3, B4, or B5 gene varies with HLA-DR type. The HLA-DQB1 gene also shows a high degree of polymorphism and thus far 25 alleles have been reported (Bodmer et al., 1995). Moreover, each HLA-DRB1 allele shows characteristic linkage disequilibria with certain alleles at the other DRB and DQB1 loci, and thus forms characteristic haplotypes.

DNA high-resolution typing methods have become available to determine HLA class II alleles. In the present study, we determined HLA class II DRB1, DRB3, and DQB1 alleles for preserved Ainu DNA samples using the polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) method.

Typing for major DRB1 groups. Genomic DNAs (Harihara et al., 1986) from 50 Ainu living in Hidaka district, Hokkaido, were typed for DRB1 groups using a PCR sequence-specific oligonucleotide (PCR-SSO) method as described in a previous report (Kimura and Sasazuki, 1992). We selected eight probes (DRB1001 and 1003–1009) among the 24 probes described in the report. This typing enabled us to classify DR alleles into eight major groups: DR1, DR2, DR4, DR7, DR9, DR10, DR52-1 (DR8, DR12, and DRB1*1404), and DR52-2 (DR3, DR11, DR13, and most DR14) (Bannai et al., 1994a).

Allele typing. High-resolution typing for DRB1 and DQB1 alleles was performed using the PCR-SSCP method described previously (Bannai et al., 1994a,b), with modifications. Group-specific amplifications for DRB1 were performed based on the results of the group typing described above. New primer sets were adopted for the samples possessing DR4- and DR52-2-group alleles in addition to those of the previous systems (Table 1). The samples with DR52-group (DR3, 11, 12, 13, 14) alleles were further typed for DRB3 alleles. The PCR-SSCP method for detecting DRB3 alleles and that for detecting DQ1-group DQB1 alleles were newly developed in this study (Table 1), us-

TABLE 1. Primer sets, alleles tested, and PCR and SSCP conditions in newly developed systems

Primer set[1]	Sense primer	Antisense primer	Alleles tested	Length (bp)	PCR	SSCP	
					Annealing-polymerization temperature (°C)	Gel concentration (%)	Electrophoretic temperature (°C)
DRB1 locus			DRB1*				
DR4/1C2	DRBAMP-4[2]	DRB8601C2[3]	0401, 0405, 0407	260	60	10	4
DR4/3C2	DRBAMP-4[2]	DRB8603C2[3]	0402, 0403, 0404, 0406, 0410, 0412	260	58	10	25
DR52-2/1C2	DR52-2A[2]	DRB8601C2[3]	1101, 1302, 1402, 1403, 1407	250	60	12.5	25
DR52-2/3C2	DR52-2A[2]	DRB8603C2[3]	0301, 1102, 1103, 1104, 1106, 1301, 1401, 1405, 1406	250	58	12.5	25
DRB3 locus			DRB3*				
DRB3	DRBAMP-52[2]	DRBAMP-B[2]	0101, 0202, 0301	271	65	10	15
DQB1 locus			DQB1*				
DQw1	GH28NL[2]	QB202[2]	0501, 0502, 0503, 0601, 0602, 0604	241	58	10	30

[1] The SSCP analyses for DR2-, DR4-, DR52-1-, and DR52-2-group DRB1 alleles and the DQw2,3,4-group DQB1 alleles were performed as previously described (Bannai et al., 1994a,b).
[2] Some of the primers were previously described (Nomura et al., 1991; Kimura and Sasazuki, 1992; Bannai et al., 1994a).
[3] Sequences of newly designed primers in this study were 5′-CTGCACTGTGAAGCTCTCAC (DRB8601C2) and 5′-CTGCACTGTGAAGCTCTCCA (DRB8603C2).

ing the standard DNAs which had been typed by PCR-SSO (Kimura and Sasazuki, 1992).

Restriction enzyme digestion. Restriction enzyme digestion was adopted for discrimination between two DR12 alleles, DRB1*1201 and 1202, because it was difficult to distinguish them by the PCR-SSCP method. One microliter of the reaction mixture containing the PCR products (from the same reaction mixture as those used for the SSCP) was digested with 0.68 U of Fok I (New England Biolabs, Beverly). Digested fragments of DRB1*1201 (160 bp and 95 bp) and the undigested fragment of DRB1*1202 (255 bp) were separated by 10% polyacrylamide gel electrophoresis and detected by silver staining (Daiichi Pure Chemicals, Tokyo).

Sequencing analysis. For sequence confirmation of the recently described allele DRB1*1106, both strands of the PCR-amplified DNA fragments were directly sequenced as described previously (Bannai et al., 1994b).

Frequency data analysis. Allele and haplotype frequencies were determined by direct counting. Eight individuals, in whom only one allele was observed at each of the DRB1, (DRB3), and DQB1 loci, were designated homozygotes. The allele numbers of homozygotes were counted double. The most probable DRB1-(DRB3)-DQB1 haplotype could be deduced in each sample based on homozygosity and linkage disequilibrium data. We also calculated the allele and haplotype frequencies by the maximum likelihood methods (Imanishi et al., 1992a), and compared them with those obtained using the direct counting method. No allele or haplotype frequency differed by more than 1% when results of the two calculation methods were compared. Estimated allele frequencies in the Ainu population were further compared with those reported for other populations (Imanishi et al., 1992b; Cerna et al., 1993).

Principal component analysis

Principal component (PC) analysis is a statistical method for condensing information concerning numerous variables into a few synthetic variables (Menozzi et al., 1978; Piazza et al., 1980). With PC analysis of allele frequencies, it is possible to measure genetic similarity and dissimilarity among ethnic groups. PC analysis was conducted using the frequencies of HLA-DRB1 and HLA-DQB1 alleles in 17 ethnic groups. The allele frequency data of South Africans,

North American Africans, French, Germans, Italians, non-Austronesian Highlanders in Papua New Guinea, Japanese, Koreans, Buyi in south China, and Tlingit in Alaska were obtained from an earlier report presented at the 11th International Histocompatibility Workshop (Imanishi et al., 1992b). The data of northern Han and southern Han were obtained from reports by Gao et al. (1991) and by Wang et al. (1993), respectively. The data of two Guaicuruan-speaking Argentinian tribes, eastern Toba and Mataco-Wichi, one Ge-speaking Brazilian tribe, Xavantes, and North American Indians (tribes undefined) were obtained from a report by Cerna et al. (1993). First, four PCs were extracted based on the variance–covariance matrix of allele frequencies. PC scores for these ethnic groups were calculated and plotted on x–y planes.

RESULTS

Major DRB1 groups

HLA-DRB1 alleles in 50 Ainu samples were first classified into eight major groups. Gene frequencies of DR1-, DR2-, DR4-, DR7-, DR9-, DR10-, DR52-1-, and DR52-2-group alleles were estimated to be 1, 3, 13, 0, 14, 0, 23, and 46%, respectively.

DRB1 and DQB1 alleles. Samples with DR2-, DR4-, DR52-1-, and DR52-2-group alleles were further subjected to high-resolution typing by the PCR-SSCP method. Figure 1 shows an example of the PCR-SSCP pattern, which demonstrates the existence of DRB1*1401, 1406, and 1106 in the Ainu population. Identical typing results were obtained using both previously and newly established PCR-SSCP conditions. Furthermore, the SSCP typing of DRB1*1106 was confirmed by nucleotide sequencing of two samples.

Estimated DRB1 allele frequencies in the Ainu population are shown in Table 2. DRB1*1401 (20%), DRB1*1406 (17%), DRB1*0901 (14%), and DRB1*0802 (10%) were observed at frequencies of 10% or more, and DRB1*1202 and DRB1*0405 were also commonly observed. Furthermore, the newly described rare allele DRB1*1106 was found in five persons (5% allele frequency). For the DQB1 locus, DQB1*0301 (37%), DQB1*0503 (20%), DQB1*0303 (14%), and DQB1*0402 (12%) were observed at frequencies of more than 10%.

On the other hand, common alleles reported in the major Japanese Wajin population (DRB1*1502, 1302, 0803, and 1501 with frequencies of 7–9%) were found at relatively low frequencies (1–2%) in the Ainu (Table 3).

Class II haplotypes. The deduced DRB1-(DRB3)-DQB1 haplotypes and their frequencies in the Ainu population are summarized in Table 2. Each DRB1 allele consisted of only one kind of haplotype. All the haplotypes observed in this study have been reported in one or more east Asian population (Imanishi et al., 1992b; Bannai et al., 1994b).

Principal component analysis

Figure 2 shows the result of PC analysis of various populations based on the allele frequencies of HLA-DRB1 and DQB1. The first four components were extracted, and contributed 44.7%, 13.0%, 10.9%, and 7.4%, respectively (76.0% cumulative), to the total variance. For the first PC (Fig. 2A), the Ainu had the sixth-highest score (0.19), following those of five Native American populations, Xavantes (2.07), Tlingit (1.71), Mataco-Wichi (1.55), eastern Toba (1.20), and North American Indians (0.77). Other Asian, Caucasian, and African populations showed considerably lower scores ranging from −0.87 to −0.38. However, in terms of the second PC (Fig. 2A), the Ainu score (−1.23) was included in the score range of other Asian populations (−1.89 to −0.18). The third and fourth PC are also shown in Figure 2B, although their contributions to the total variance were less. The Ainu were close to some Native American populations for the third PC and close to east Asian and some Native American populations for the fourth PC. On the whole, the Ainu did not constitute a cluster with any other ethnic group in this analysis. On the contrary, in terms of PC analysis, the Ainu were situated midway between Native Americans and a cluster of east Asian populations including Japanese (Wajin).

DISCUSSION

Recent morphological and genetic studies have supported the hypothesis that the

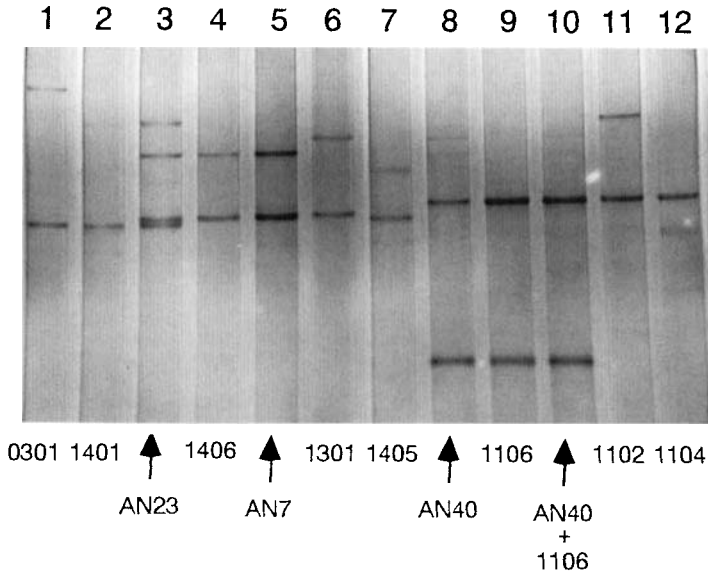

Fig. 1. PCR-SSCP analysis of HLA DR52-2/3C2-group DRB1 alleles. The primer set DR52-2A and DRB8603C2 was used for amplifications. The alleles of standard samples are as follows: **lanes 1, 2, 4, 6, 7, 9, 11,** and **12** are DRB1*0301, 1401, 1406, 1301, 1405, 1106, 1102, and 1104, respectively. AN23 **(lane 3),** AN7 **(lane 5),** and AN40 **(lane 8)** are typed as DRB1*1401/1406, 1406, and 1106, respectively. **Lane 10** is a mixture of AN40 and DRB1*1106 standard.

TABLE 2. DRB1 allele and class II haplotype frequencies in Ainu

DRB1	DRB3	DQB1	Frequency (%)	Standard error (%)
01	—	0501	1.0	1.0
1501	—	0602	2.0	1.4
1502	—	0601	1.0	1.0
1101	0202	0301	3.0	1.7
1106	0202	0301	5.0	2.2
1201	0101	0301	4.0	2.0
1202	0301	0301	8.0	2.7
1302	0301	0604	1.0	1.0
1401	0202	0503	20.0	4.0
1406	0202	0301	17.0	3.8
0403	—	0302	3.0	1.7
0405	—	0401	8.0	2.7
0410	—	0402	2.0	1.4
0802	—	0402	10.0	3.0
0803	—	0601	1.0	1.0
0901	—	0303	14.0	3.5
Total			100.0	

Wajin, or Hondo-Japanese, who constitute the major population of contemporary Japan, are descendants mainly of postneolithic migrants who traveled from the east Asian continent in the Yayoi (300 BC–300 AD) and the Kofun (300–600 AD) eras. The Ainu of Hokkaido (the northernmost island of Japan) as well as the Ryukyuans of Okinawa (southern islands of Japan), although now mixed with Wajin, are considered to be the descendants of preagricultural indigenous populations of Japan (Jomonese) (Omoto, 1972, 1973; Omoto and Misawa, 1976; Ikeda, 1982; Hanihara, 1984, 1991; Ishida et al., 1985). In a previous study (Omoto, 1973), the admixture rate of Ainu high-school students in the Hidaka district was estimated to be 40% based on data obtained from official family records and interviews. The admixture rate in the samples of the present study is presumed to be lower, because only adults were included in the samples. Although there has been considerable gene flow from non-Ainu Japanese into Ainu, the study of HLA polymohphism should clarify the relationship between Ainu and non-Ainu Japanese and other ethnic groups who live in areas neighboring Japan.

In the present study, the principal component analysis of HLA DRB1 and DQB1 allele frequencies showed that the Ainu are situated midway between Native American and

TABLE 3. DRB1 allele frequencies in Ainu as compared with those in other populations[1]

	East Asian					Native American				European		African
	Ainu	Wajin	Korean	Singapore Chinese	Buyi	Zuni	Tlingit	Eastern Toba	Xavantes	Italian	Spanish Gypsy	San (Bushman)
DRB1* 1401	20.0 (4.0)	3.8	1.5	2.1	16.9	0.0	0.9	0.4	0.0	5.1	30.3	1.3
DRB1* 1406	17.0 (3.8)	1.7	0.5	0.0	0.0	9.0	0.0	23.7	0.0	0.0	0.0	0.0
DRB1* 0901	14.0 (3.5)	13.7	9.0	15.4	8.3	2.0	4.7	1.1	0.0	0.2	1.4	0.6
DRB1* 0802	10.0 (3.0)	3.8	0.5	0.0	0.0	21.0	0.9	22.3	26.5	0.2	0.0	3.9
DRB1* 1202	8.0 (2.7)	2.0	2.5	8.4	8.3	0.0	1.9	0.0	0.0	0.0	0.0	0.0
DRB1* 0405	8.0 (2.7)	12.5	7.0	7.1	2.1	0.0	0.0	0.4	0.0	0.0	0.0	0.0
DRB1* 1106	5.0 (2.2)	0.0	rare	rare	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DRB1* 1502	1.0 (1.0)	9.2	3.0	0.7	4.3	0.0	0.0	0.0	0.0	2.2	8.0	0.0
DRB1* 1302	1.0 (1.0)	7.4	12.5	0.0	0.0	0.0	1.9	0.0	0.0	4.0	4.1	12.0
DRB1* 0803	1.0 (1.0)	7.3	7.5	5.7	2.9	0.0	0.0	0.0	0.0	0.5	0.0	0.0
DRB1* 1501	2.0 (1.4)	6.8	8.0	13.3	15.4	0.0	5.7	0.4	0.0	5.5	0.7	0.6

[1]Allele frequencies (%) in other populations were obtained from summary reports of the 11th International Histocompatibility Workshop (Imanishi et al., 1992b) and another report (Cerna et al., 1993). The upper panel shows the frequencies (%) of alleles commonly observed in Ainu. The lower panel shows the frequencies (%) of alleles observed relatively infrequently in Ainu but commonly observed in Wajin. Standard error for each allele frequency (%) of Ainu is shown in parentheses.

east Asian populations. This result indicates that the Ainu are genetically relatively distantly related to the other Asian populations.

It should be noted that a small tribe sometimes appears to be distantly related to closely related ethnic groups for certain reasons. The present data showed a limited number of common alleles; 51% of DRB1 variation is accounted for by only three different alleles (DRB1*1401, 1406, and 0901). A similar situation was observed in Native American tribes, and possible reasons which have been proposed include a small number of founder individuals, selective advantage of some alleles in terms of resistance to illness, or random genetic drift in the gene pool in isolated tribes (Cerna et al., 1993). These genetic factors could affect the principal component analysis results.

However, the distribution data of each allele commonly observed in the Ainu also indicate that the Ainu are situated midway between Native American and east Asian populations (Table 3). First, DRB1*1406 was observed at high frequency (17%) in the Ainu. This allele has not been found in southern Chinese, Europeans, or Africans, and has been infrequently observed in Japanese and Koreans, but is common in some Native Americans such as the North American Zuni and Argentinian Indians (Imanishi et al., 1992b; Cerna et al., 1993). A similar distribution has been observed for another allele, DRB1*0802, which has been reported to be common in Native Americans but rare in southern Chinese or Europeans (Imanishi et al., 1992b; Cerna et al., 1993). Second, other alleles show distinct distribution patterns. A rare allele, DRB1*1106, previously found only in two Singapore Chinese and one Korean (Lin et al., 1993; Bannai et al., 1994b), was found to be common (5%) in the Ainu. DRB1*1106 seems to be more than a "private" polymorphism among the Ainu. DRB1*1202 is common in Asian populations including Ainu but rare in native Americans. DRB1*1401 is also common in Ainu but rare in Native Americans. These data further support the hypothesis that the Ainu are somewhat distantly related to both Native Americans and present-day east Asian populations.

The haplotype data were consistent with the allele data. DRB1*1106 was found in the present study as part of the haplotype DRB1*1106-DRB3*0202-DQB1*0301, which coincides with that identified in a Korean (Bannai et al., 1994b). DRB1*1406 was present in the haplotype DRB1*1406-DRB3*0202-DQB1*0301 in the Ainu, which is the same as one reported to be present in the majority of the Japanese population (Imanishi et al., 1992b). In Native Americans, however, this allele is included in different haplotypes, DRB1*1406-DRB3*0101-DQB1*0301 (Fernandez-Viña et al., 1991; Cerna et al., 1993) or DRB1*1406-

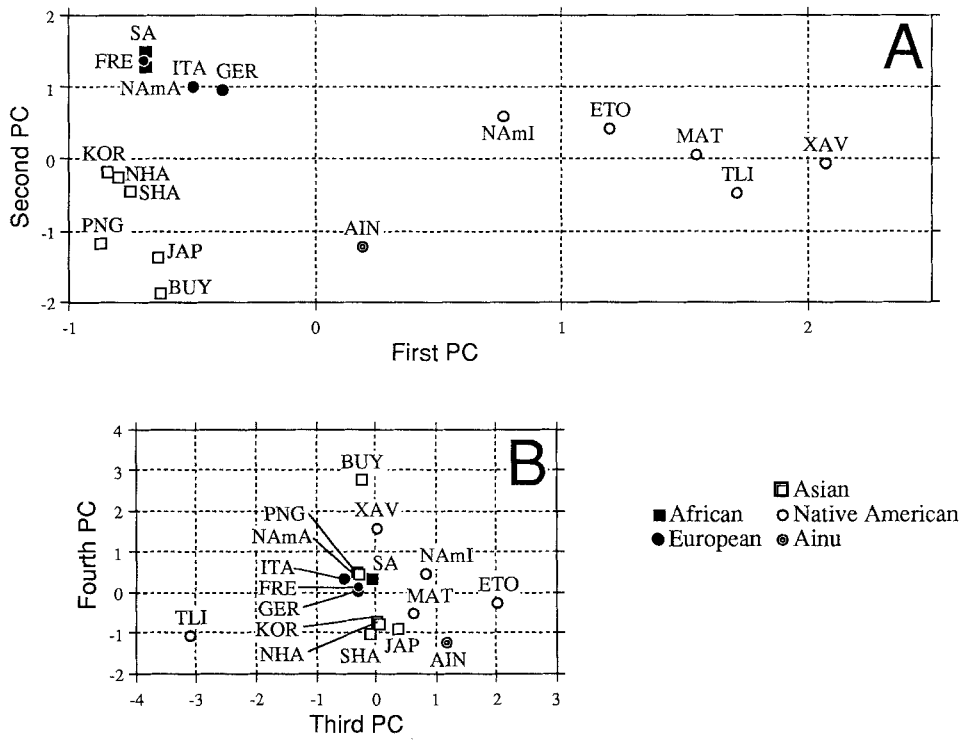

Fig. 2. Principal component (PC) analysis showing relationships between the Ainu and other ethnic groups. The following 17 populations were compared: South Africans (SA), North American Africans (NAmA), non-Austronesian Highlanders in Papua New Guinea (PNG), French (FRE), Germans (GER), Italians (ITA), Japanese (JAP), Koreans (KOR), Buyi in south China (BUY), Ainu (AIN), northern Han (NHA), southern Han (SHA), Tlingit in Alaska (TLI), eastern Toba in Argentina (ETO), Mataco-Wichi in Argentina (MAT), Xavantes in Brazil (XAV), and North American Indians (NAmI). Contributions of the first, second, third, and fourth PCs are 44.7%, 13.0%, 10.9% and 7.4%, respectively (76.0% total). Each axis is sized in proportion to the contribution. **A:** The first PC (abscissa) plotted against the second PC (ordinate). **B:** The third PC (abscissa) plotted against the fourth PC (ordinate).

DRB3*0101-DQB1*0302 (Moraes et al., 1993). This difference may have resulted from a recombination(s) occurring after the migration of Native American ancestors from Asia to America.

Several common alleles in the Wajin were found at low frequencies in the Ainu. Each of DRB1*1502 and 1302 (9.2% and 7.4% in Wajin, respectively) were found only once in the Ainu. DRB1*1502 is included in the predominant haplotype HLA-A24-B52-DR15-DQ6 in Wajin (Tokunaga et al., 1985, 1996; Imanishi et al., 1992b; Akaza et al., 1994), and the B52-DR2(15) haplotype is also common in Mongolian, northern Han Chinese, and Korean populations (Tokunaga et al., 1996). DRB1*1302 is included in the second-most common haplotype, HLA-A33-B44-DR13-DQ6, in Wajin (Tokunaga et al., 1985, 1996; Imanishi et al., 1992b; Akaza et al., 1994), and the B44-DR13 haplotype is predominant in the Korean population. These alleles and haplotypes may have been carried by migrants who entered Japan after the neolithic Yayoi period (Tokunaga et al., 1996).

Genetic markers other than HLA genes have also been studied by means of DNA analysis in the Ainu. Harihara et al. (1986) analyzed mitochondrial DNA polymorphism and reported that their results supported the hypothesis that the Ainu are of "Mongoloid"

origin. Recently Miura et al. (1994) phylogenetically analyzed genomic sequences of human T-lymphotropic virus type I (HTLV-I) and reported that a subtype found from one Ainu and one Asian Indian individual was also found in populations in the Caribbean basin and South America.

These data and our findings indicate that the Ainu are a unique "Mongoloid" population and genetically related to both Native American and east Asian populations. It is also suggested that the Ainu are probably descended from some Upper Paleolithic populations of east Asia who genetically diverged from the present-day east Asian "Mongoloids" before the advent of the neolithic period.

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LITERATURE CITED

Akaza T, Imanishi T, Fujiwara K, Tokunaga K, Yashiki S, Fujiyoshi T, Sonoda S, and Juji T (1994) HLA alleles and haplotypes of Japanese population. MHC IRS *1:*219–226.

Bannai M, Tokunaga K, Lin L, Kuwata S, Mazda T, Amaki I, Fujisawa K, and Juji T (1994a) Discrimination of human HLA-DRB1 alleles by PCR-SSCP (single-strand conformation polymorphism) method. Eur. J. Immunogenet. *21:*1–9.

Bannai M, Tokunaga K, Lin L, Park MH, Kuwata S, Fujisawa K, and Juji T (1994b) A new HLA-DR11 DRB1 allele found in a Korean. Hum. Immunol. *39:*230–232.

Bodmer JG, Marsh SGE, Albert ED, Bodmer WF, Bontrop RE, Charron D, Dupont B, Erlich HA, Mach B, Mayr WR, Parham P, Sasazuki T, Schreuder GMTh, Strominger JL, Svejgaard A, and Terasaki PI (1995) Nomenclature for factors of the HLA system, 1995. Tissue Antigens *46:*1–18.

Cerna M, Falco M, Friedman H, Raimondi E, Maccagno A, Fernandez-Viña M, and Stastny P (1993) Differences in HLA class II alleles of isolated South American Indian populations from Brazil and Argentina. Hum. Immunol. *37:*213–220.

Fernandez-Viña M, Moraes JR, Moraes ME, Miller S, and Stastny P (1991) HLA class II haplotypes in Amerindians and in black North and South Americans. Tissue Antigens *38:*235–237.

Gao X, Sun Y, An J, Fernandez-Viña M, Qou J, Lin L, and Stastny P (1991) DNA typing for HLA-DR, -DQ, and -DP alleles in a Chinese population using the polymerase chain reaction (PCR) and oligonucleotide probes. Tissue Antigens *38:*24–30.

Hanihara K (1984) Origins and affinities of Japanese viewed from cranial measurements. Acta Anthropogenet. *8:*149–158.

Hanihara K (1991) Dual structure model for the population history of the Japanese. Jpn. Rev. *2:*1–33.

Harihara S, Hirai M, and Omoto K (1986) Mitochondrial DNA polymorphism in Japanese living in Hokkaido. Jpn. J. Hum. Genet. *31:*73–83.

Ikeda J (1982) Interpopulation variations of the frontal sinus measurements. J. Anthropol. Soc. Nippon *90:*91–104 (in Japanese with English summary).

Imanishi T, Wakisaka A, and Gojobori T (1992a) Genetic relationships among various human populations indicated by MHC polymorphisms. In K Tsuji, M Aizawa, and T Sasazuki (eds.): HLA 1991, I. Oxford, Oxford University Press, pp. 627–632.

Imanishi T, Akaza T, Kimura A, Tokunaga K, and Gojobori T (1992b) Allele and haplotype frequencies for HLA and complement loci in various ethnic groups. In K Tsuji, M Aizawa, and T Sasazuki (eds.): HLA 1991, I. Oxford, Oxford University Press, pp. 1065–1220.

Ishida T, Yamamoto K, Omoto K, Iwanaga M, Osato T, and Hinuma Y (1985) Prevalence of a human retrovirus in native Japanese: Evidence for a possible ancient origin. J. Infection *11:*153–157.

Kimura A, and Sasazuki T (1992) Eleventh International Histocompatibility Workshop reference protocol for the HLA DNA-typing technique. In K Tsuji, M Aizawa, and T Sasazuki (eds.): HLA 1991, I. Oxford, Oxford University Press, pp. 397–419.

Lin YN, Ren EC, and Chan SH (1993) A new DR11 allele in Singaporean Chinese. Tissue Antigens *41:*204–205.

Menozzi P, Piazza A, and Cavalli-Sforza L (1978) Synthetic maps of human gene frequencies in Europeans. Science *201:*786–792.

Misawa S, Hayashida Y, Miki T, Omoto K, Harada S, Matsumoto H, Komatsu I, and Hirai M (1975) Genetic composition of the Ainu. In S Watanabe, S Kondo, and E Matsunaga (eds.): JIBP Synthesis, II. Anthropological and Genetic Studies on the Japanese. Tokyo, University of Tokyo Press, pp. 265–307.

Mittal KK, Hasegawa T, Ting A, Mickey MR, and Terasaki PI (1973) Genetic variation in the HL-A system between Ainus, Japanese, and Caucasians. In J Dausset and J Colombani (eds.): Histocompatibility Testing 1972. Copenhagen, Munksgaard, pp. 187–195.

Miura T, Fukunaga T, Igarashi T, Yamashita M, Ido E, Funahashi S, Ishida T, Washio K, Ueda S, Hashimoto K, Yoshida M, Osame M, Singhal BS, Zaninovic V, Cartier L, Sonoda S, Tajima K, Ina Y, Gojobori T, and Hayami M (1994) Phylogenetic subtypes of human T-lymphotropic virus type I and their relations to the anthropological background. Proc. Natl. Acad. Sci. USA *91:*1124–1127.

Moraes ME, Fernandez-Viña M, Salatiel I, Tsai S, Moraes JR, Stastny P (1993) HLA class II DNA typing in two Brazilian populations. Tissue Antigens *41:* 238–242.

Nomura N, Ota M, Tsuji K, and Inoko H (1991) HLA-DQB1 genotyping by a modified PCR-RFLP method combined with group-specific primers. Tissue Antigens *38:*53–59.

Omoto K (1972) Polymorphisms and genetic affinities of the Ainu of Hokkaido. Hum. Biol. Oceania *1:*278–288.

Omoto K (1973) The Ainu: A racial isolate? Isr. J. Med. Sci. *9*:1285–1290.

Omoto K, and Misawa S (1976) Genetic relations of the Ainu. In RL Kirk and AG Thorne (eds.): Biological Origins of the Australians. Canberra, Australian Institute Aboriginal Studies, pp. 365–376.

Piazza A, Menozzi P, and Cavalli-Sforza LL (1980) The HLA-A, B gene frequencies in the world: Migration or selection? Hum. Immunol. *4*:297–304.

Tokunaga K, Omoto K, Akaza T, Akiyama N, Amemiya H, Naito S, Sasazuki T, Satoh H, and Juji T (1985) Haplotype study on C4 polymorphism in Japanese. Associations with MHC alleles, complotypes, and HLA-complement haplotypes. Immunogenetics *22*: 359–365.

Tokunaga K, Saueracker G, Kay PH, Christiansen FT, Anand R, and Dawkins RL (1988) Extensive deletions and insertions in different MHC supratypes detected by pulsed field gel electrophoresis. J. Exp. Med. *168*: 933–940.

Tokunaga K, Imanishi T, Takahashi K, and Juji T (1996) On the origin and dispersal of East Asian populations as viewed from HLA haplotypes. In T Akazawa and EJE Szathmary (eds.): Prehistoric Mongoloid Dispersals. Oxford, Oxford University Press, pp. 187–197.

Wang FQ, Semana G, Fauchet R, and Genetet B (1993) HLA-DR and -DQ genotyping by PCR-SSO in Shanghai Chinese. Tissue Antigens *41*:223–226.